

ABSTRACT

THESIS: CREATING CHIMERAS OF HUMAN G - PROTEIN COUPLED RECEPTORS (HGPR40/43) FOR DIABETIC DRUG DEVELOPMENT

STUDENT: DEEPAK ACHARYA

DEGREE: Master of Science

COLLEGE: Sciences and Humanities

DEPARTMENT: Biology

DATE: February, 2009

PAGES: 63

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder of global concern that is primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia. G-Protein Coupled Receptors (GPCRs), important mediators of cellular signaling responses, have been prime targets of drug discovery efforts in various therapeutic areas. Human G-Protein Receptor 40 (HGPR40) is highly expressed in the pancreas and has been implicated in the regulation of glucose metabolism and pathophysiology of T2DM. For effective control of diabetes, combination therapy is being considered because no single drug can completely control diabetes and its associated complications. This necessitates the identification of novel drug targets including HGPR40 which might permit development of drugs which function differently from

existing drugs. This project focused on the construction of two chimeric receptor proteins named HGPR40.1-715_43.709-1013 (Chimera I) and HGPR40.1-431_43.389-1013 (Chimera II) which were made by exchanging domains within trans-membranes regions 6 and 4, of HGPR40 and HGPR43 DNA, homologous receptors which vary in amino acid sequence but which have the same three-dimensional structure. After PCR amplification of sufficient quantities of the desired gene fragments they were ligated together to form the desired recombinant chimeric proteins which were cloned into the expression vector pcDNA3.1 in two successive cloning steps. The vector contains CMV promoter, multiple cloning sites, neomycin resistance gene etc for high-level expression in a wide range of mammalian cells. The two full-length chimeras were designed to be 6452 bp and 6483 bp by exchanging either the first or the first two external domains of HGPR40 with those of HGPR43. The creation of the correct chimeras was verified by both agarose gel electrophoresis and PCR analysis. Then chimeric DNA was transformed into *Escherichia coli* to obtain sufficient amounts of DNA for sequencing verification of the desired construct. Upon verification, the cloned DNA was to be transfected into cultured mammalian kidney (HEK293) cells for expression of the chimeric proteins. A (FLIPR) Fluorometric Imaging Plate Reader analysis by our collaborators at Eli Lilly would have been used to measure the extent of Ca^{2+} efflux from the endoplasmic reticulum testing a variety of stimulatory molecules to obtain an indication of which would activate the receptor. Activation would initiate the internal (second messenger cascade) G-protein signaling pathway and result in the secretion of insulin. However, transformation of the chimeric membrane receptors into *E. coli* resulted in altered sequences which could not be used for the FLIPR analysis. Expression of membrane proteins in *E. coli* can lead to such gene rearrangements. These experiments will be repeated in the future using another type of competent cell designed to prevent genetic re-arrangements. Construction of stable clones will permit us to test numerous potential ligands to aid in development of novel therapeutic drugs targeting HGPR40 to aid in combating diabetes.

